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Renal Cell Carcinoma

Cytogenetic Analysis of Tumors and Cell Lines

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ABSTRACT: *Successful cytogenetic analysis was performed on 27 samples from 25 patients with RCC, including 7 of 11 tumors studied and 20 cell lines. Clonal chromosomal abnormalities were detected in all 27 samples. The most frequently involved chromosomes were 7, 1, 3, 9, and the Y (20, 17, 17, 14, and 10 cases, respectively). Polysomy 7 or rearrangement of 7q was seen in 80% (20/25) of the patients, and loss or rearrangement of 3p was seen in 48% (12/25); of the latter, four patients had loss of the whole chromosome and 10 patients had deletions or translocations involving 3p, with breakpoints at either 3p11–14 or 3p21–23 (5/7 translocation breakpoints were at 3p21–23). Loss of the sex chromosomes was seen in 15 patients, including −Y in 10/22 males. Other clonal changes included structural abnormalities of chromosome 1 centromere and the long arm, breakpoints at or near the centromere of chromosome 9 (10 patients), polysomy 16, monosomy 17, polysomy 20, and monosomy 22. With the exception of chromosome 3p loss, which was primarily confined to the nonpapillary cases, no specific clonal abnormality was noted for any particular subtype of RCC. Trisomy or tetrasomy 7 and −Y were seen in all subtypes of renal cell carcinoma.*

INTRODUCTION

Renal cancer accounts for approximately 3% of all malignancies. An estimated 27,000 new cases are diagnosed each year, with about 10,300 deaths annually. The disease shows a 2:1 male predominance and the average age at diagnosis is 55–60 years. The etiology of RCC is obscure; the growth patterns of the primary tumor are variable and may remain localized for years, but the increased usage of imaging modalities and technologic improvements in their quality has led to significantly increased early detection of clinically asymptomatic cases. The histologic variants of RCC include clear cell (80% of tumors), granular and sarcomatoid cell (about 10% of tumors), and papillary (8–14% of tumors) [1, 2].

The most recurrent cytogenetic patterns reported in RCC are aberrations of chromosome 3 and polysomy of chromosome 7. Rearrangements of 3p have been found in both hereditary [3–5] and sporadic forms of the disease [5–10], indicating that loss of the 3p segment plays a critical role in the development of RCC. The breakpoints cluster in region 3p11–p21, usually at 3p14, and restriction fragment length polymorphism (RFLP) analysis using molecular probes for 3p14–p21 has shown that at least one allele is missing in most RCCs [11]. Although few of the cytogenetic studies published have distinguished between the various histologic subtypes of RCC, Kovacs et al. [12] reported that while deletion of 3p is present in more than 90% of cases of nonpapillary RCC, papillary tumors fail to show 3p rearrangement on a cytogenetic or molecular level. The occurrence of trisomy 7, alone or in combination with other chromosome abnormalities, has led to speculation that chromosome 7 may confer a growth advantage to malignant cells, especially since the epidermal growth factor receptor has been mapped to 7p [13, 14].

To better understand the genetics of renal cell carcinoma we undertook a study of cytogenetic aberrations in tumor tissue from 25 patients, correlating the results with tumor cytologic type and known chromosome 3p deletions as previously assessed by RFLP analyses.

MATERIALS AND METHODS

Normal and tumor tissue samples were obtained immediately after resection from 25 patients who had surgery at the Surgery Branch of the National Cancer Institute. Cytogenetic studies were performed on a total of 27 samples. Direct and short-term culture preparations were attempted in 11 patients, seven of which were successful; cultured cell lines were successfully analyzed from 19 patients, including one of the patients with successful direct studies. There were 22 male and three female patients; their ages ranged from 21 to 67 years, with a median age of 53 years. Tumor location was on the left side in 13 patients, on the right side in 10 patients, and

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Received June 8, 1994; accepted January 27, 1995.

Cancer Genet Cytogenet 82:128–139 (1995)

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0165-4608/95/$09.50
SSDI 0165-4608(95)00024-J

Table 1 Cytogenetic studies in 25 patients with renal cell carcinoma

Pt. no.	Sex/age	Pathologic diagnosis	Chromosomal no. mode (range)	Clonal chromosome abnormalities
Papillary				
1	M/67	Papillary	48 (33–37)	51,XY, + i(1)(q10), + 4, + 7, + 7, – 8, + 16, + 17, – 18,der(19)t(2;19)(q21;q13), + 21
2[a]	M/60	Papillary	50 (41–104)	46,X, – Y,der(2)t(1;2)(q25;q37), + del(6)(q13), + 7, + 7,der(15)t(3;15)(q11;p11), + 20
3	F/21	Papillary	68,70 (46–154)	69 < 3n >,X, – X, – X,der(1)t(1;17)(q21;q21), + del(1)(q32), + 2, – 3, del(6)(q21), + del(6)(q13), + 7, + del(7)(q11),del(9)(q11),del(10)(p12), – 10, – 12,del(13)(q14),del(14)(q22), – 15, – 17, – 17, – 18, + 19, + 20
16	M/39	Papillary	50 (34–50)	50,XY, + 2, + der(7)t(7;8)(q36;q22)x2,del(7)(q22),i(8)(q10), + der(11)t(9;11)(q11;p11)
		Metastatic tumor	46,47 (36–50)	48,XY, + del(1)(p11), + 2, + del(3)(p12), – 5,del(7)(p11), + i(8)(q10), t(9;11)(q11;p11)
17[a]	M/63	Papillary	50 (38–52)	50,X, – Y, + t(1;10)(p11;q11), + der(4)t(4;7;11;5)(:4p12→4q13::7p11→7pter::11q13→11q25::5q13→5qter), – 5, + 7, + 7, – 10, + 16, + del(17)(p12), + 20
		Cell line	50 (45–54)	51,X, – Y, + der(4)t(4;7;11;5)(:4p12→4q13::7p11→7pter::11q13→11q25::5q13→5qter), + i(4p), – 5, + 7, + 7, + 16, + del(17)(p12), + 20
21	M/?	Papillary	78 (39–83)	80 < 3n >,XX, – Y,del(1)(q32), + del(1)(p31), + 2, + 3, + 4, + 5, + 6, + t(6;7)(p11;p11), + 7, – 8, + t(9;15)(p11;q11)x2, + t(7;9)(q11;q22), – 11, + 12, + 12, + 13,i(14q), – 15, + der(16)t(15;16)(q11;q11)x3, – 17, – 18, + 20, – 21, – 22
9	M/56	Clear/granular	73 (73–162)	73 < 3n >,XX, – Y, + 2, + der(3)t(3;7)(p21;q11), + 6, + 7, + 7,i(8)(p10), i(8)(q10)i(9)(q10), + del(10)(q22), + 12, – 13, + 16, + 16, – 17, – 18, + 19, + 20, – 21, – 22
12	M/42	Clear/granular	50 (35–103)	51,XY, + X, + der(7)t(3;7)(p21;q36), + 13, + 16, + 19
14[a]	M/53	Clear/granular	49 (46–120)	50,X, – Y, + 1, + 1,t(3;8)(p14;q13), + 5, + 7, + 9, – 10, – 10, + del(11)(q13), + 12, + del(13)(q14)x2, – 14, – 17, + 20, – 21
18	M/57	Clear/granular	44 (34–74)	44,X, + X, – Y, + 7, – 8, – 9, – 13, – 15, + r
19	M/56	Clear/granular	46 (41–121)	46,XY, + del(1)(q12), + del(1)(q21), – 2, + del(6)(q14), + 6, + 14, + 15, – 17,t(12;19)(q13;q13), – 21, – 22
20	M/41	Clear/granular	77 (60–85)	77 < 3n >,XY, – X, + del(1)(q11), + del(2)(p11), + dup(2)(q11q37), – 3, + 4, + 4,t(3;5)(p21;q33),del(5)(p12), + del(6)(q21)x2,del(6)(q15), + 7, – 8, – 8,i(10q), – 11, – 13, – 14, – 15, + 16, + 16, + 17, + 19, + 20, + 20, + 21
22	M/47	Clear/granular	45 (38–83)	45,X, – Y,del(3)(p14p23),t(9;17)(p24;q21)
7	M/62	Clear/gran/sarc	53 (42–82)	77 < 3n >,XY,del(X)(q22),t(1;4)(p34;q13),der(2)t(2;15)(q37;q21), + 3, + 5, + 7, + der(7)t(7;8)(p11;q11), – 8, – 10, + 12, + 12, – 13, – 14, + 16, + der(17)t(10;17)(q11;p13), + 19, + 19, + 20, + 20
23	M/?	Gran/clear/sarc	78 (35–91)	80 < 3n >,XY, – X, + Y, + del(1)(p22)x2, – 2, + 6, + 7, + i(9)(q10), + i(9)(p10), + 9, + 11, + 13,del(14)(q24)x2,t(13;14)(q11;p11), – 15, + 16, + 18, + t(1;19)(q21;p13), + 20
24	M/50	Clear/gran/sarc	57 (54–82)	60 < 3n >,XXY,del(1)(q22), – 1,inv(2)(p23q31),del(3)(p13),del(3)(:p14q21:),del(4)(q21),t(3;4)(p13;q35), – 5, + del(7)(q21),del(7)(p11), + der(9)t(1;9)(p11;p11)x2,t(3;9)(q21;p11q34),t(9;15)(p11;q11), – 10, – 11, – 11,der(11)t(7;11)(p11;p15), – 12,t(12;13)(p13;q21), – 13, – 14, + 15, – 16, + 17, – 18, – 19, – 20, – 20, – 22

[a] Direct sample.

unknown in two patients. Tumor morphology consisted of the following pathologic diagnoses: clear cell, eight patients; clear and granular mixed cell, seven patients; clear, granular, and sarcomatoid cell, three patients; and papillary, seven patients. Cytogenetic analysis was also performed on a metastatic lung lesion from one patient with a papillary tumor.

Direct and Short-Term Cultures

A total of 14 tumor samples from 11 patients were processed directly or cultured short-term (usually less than 2 weeks), according to standard cytogenetic techniques for tumor cells. Cultures were set up in both T-flasks (25 cm^2 and 75 cm^2) and single-well chamber slides.

Monolayer Cell Cultures

Long-term cell cultures were established from 19 patients, as previously described [15]. Following surgical removal, the tumor material was minced and treated overnight with collagenase, and then washed. The fragments were dispersed in culture medium (DMEM solution) supplemented with 10–20% fetal bovine calf serum, Hepes, glutamine,

penicillin-streptomycin, and gentamycin, at a final cell concentration of 1 × 10^6/mL per medium. The cells became adherent within 24 hours and, in most cases, grew to near-confluency within 1 week after plating.

Chromosome Analysis

Most primary cultures were harvested after 3–14 days in culture, the optimum time usually being 3–8 days; a few specimens required considerably longer culture, the longest being about 35 days. All specimens were harvested according to standard techniques (some cultures required overnight treatment with Colcemid to obtain sufficient numbers of metaphases for analysis). Slides were stained with trypsin-Giemsa stain and karyotypes were prepared according to the ISCN Guidelines for Cancer Cytogenetics [16].

RESULTS

Cytogenetics

The chromosome findings in the 25 successfully studied patients with renal cell carcinoma (RCC) are summarized in Table 1. The modal chromosome number ranged from near-diploidy to near-tetraploidy: 16 patients (64%) had numbers in the diploid range (35–57 chromosomes), five patients (20%) had numbers in the triploid range (58–80), and four patients (16%) had numbers in the tetraploid range. Sex chromosome abnormalities are shown in Table 2; 60% (15 patients) of the tumors displayed missing sex chromosomes (Figs. 1–5). Two of the three female patients were missing an X chromosome: one patient was missing one X chromosome and the other was missing two X chromosomes (in triploid cells). Three males were missing an X chromosome (in polyploid cells), while three males gained an X chromosome. Loss of the Y chromosome was seen in 10 of the 22 male patients. Structural abnormality of the sex chromosomes was seen only once, a deletion of Xq in one male patient.

Breakpoints for structural abnormalities are shown in Figure 6. Chromosome 1 aberrations are shown in Table 3. A total of 17 of the 25 patients demonstrated either numerical and/or structural abnormalities of chromosome 1. Numerical abnormalities included trisomy 1 (two patients), tetrasomy 1 (one patient, Figure 5), and loss of chromosome 1 (two patients). Structural abnormalities of chromosome 1 included deletion of either the short or the long arm of chromosome 1 (10 patients) and translocations involving chromosome 1 (seven patients) (Figs. 1 and 3). No unique chromosome 1 abnormality was seen in these tumors.

A total of 17 patients had abnormalities involving chromosome 3 (Table 4), including loss or gain of the whole chromosome (eight patients) (Fig. 3), terminal or interstitial deletion of the short arm without translocation (five patients), and translocation resulting in deletion of 3p (seven patients) (Figs. 4 and 5); several patients exhibited more than one 3p abnormality. A majority of the breakpoints occurred at segment 3p13–21 (Fig. 6). Of the five patients with 3p deletions, four had breakpoints at 3p11–14 and four had breakpoints at 3p21–23 (two of the five had interstitial deletions with two breakpoints on 3p); translocations involving 3p were observed in two patients at 3p13–14 and in five patients at 3p21. Of the latter five patients, two had 3;5 translocations involving 5q33 and 5q35, and three patients had 3;7 translocations, involving 7q36 in two patients and 7q11 in one patient (Fig. 4); all five patients had clear or clear/granular RCC. Of the 12 patients showing monosomy 3 or structural involvement of 3p, four had clear cell, five had clear/granular cell, two had papillary, and one had clear/granular/sarcomatoid RCC.

Chromosome 7 abnormalities, shown in Table 5, were observed in 20 of the 25 patients (Figs. 1–4). Trisomy or tetrasomy 7 was noted in 15 patients: in 10 of the 18 non-papillary and five of the seven papillary cell cases. Deletions and/or translocations of chromosome 7 was observed in 12 patients (Figs. 2 and 4), with breakpoints clustering around the centromere region and the terminal portion of the long arm (Fig. 6).

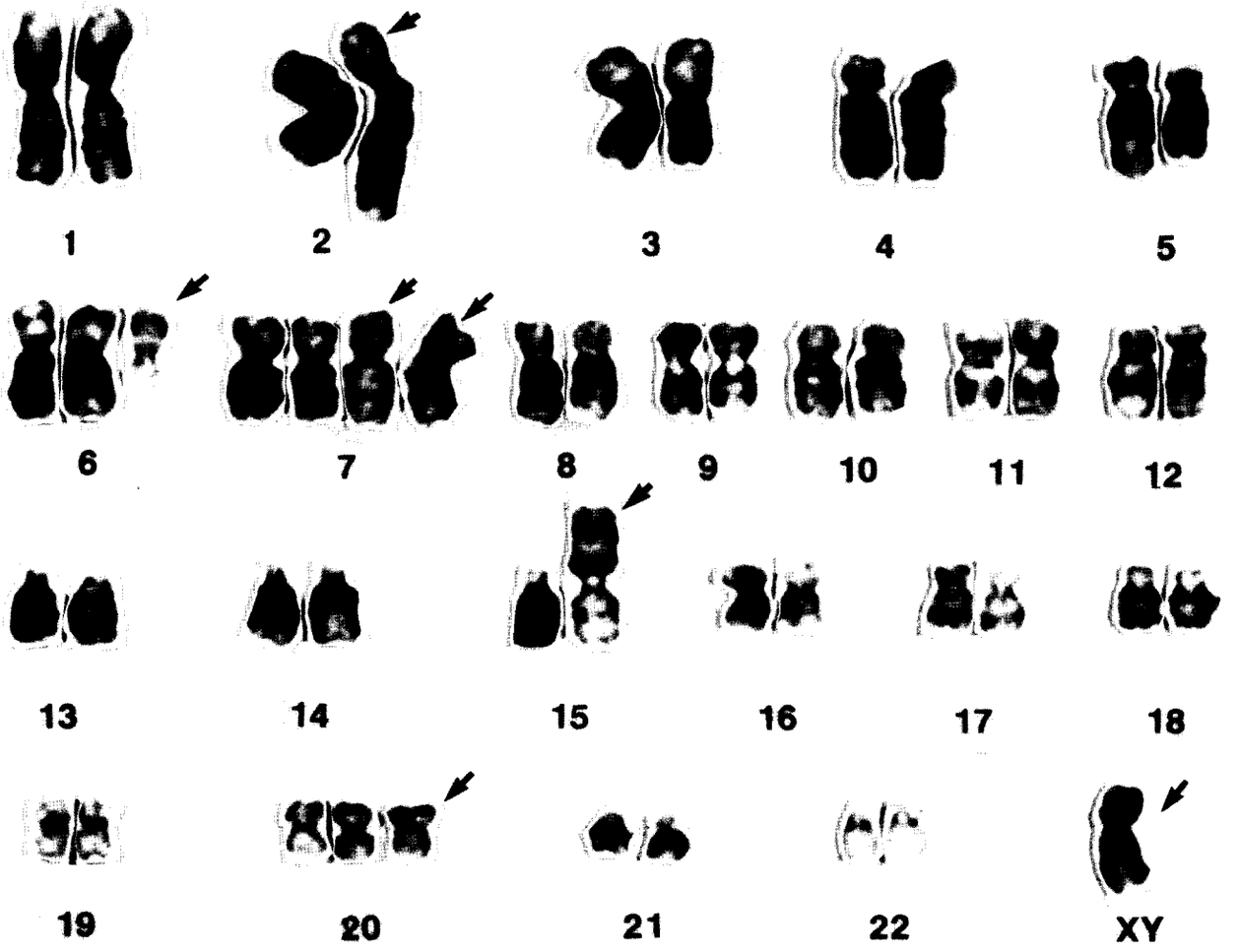
Chromosome 9 abnormalities, which clustered around the centromere region (p11 and q11), were observed in 14 patients, four of whom demonstrated isochromosome 9p or 9q (Fig. 4). Isochromosomes were found in 11 cases (Table 6), the most frequent being isochromosome of 8q (four cases) (Fig. 4).

The other chromosomes involved in numerical and structural abnormalities in these 25 patients included: chromosome 13 (four patients, all with structural abnormalities in the q14–21 region) (Fig. 5), chromosome 16 (12 patients: five had trisomy, four had tetrasomy, two had monosomy, and one had a translocation at 16q11) (Figs. 2–4), chromosome 17 (nine patients had monosomy [Fig. 5], three patients had trisomy, and two patients had tetrasomy). A high incidence of numerical abnormalities was also noted with chromosome 20 (13 patients, of whom 11 had extra chromosomes) (Figs. 1, 2, 4, and 5), and chromosome 22 (seven patients, six of whom had monosomy 22) (Fig. 4).

Table 2 Sex chromosome aberrations in RCC

	Papillary	Clear	Clear/granular	Clear/granular/sarcomatoid
Total no. pts./subgroup	7	8	7	2
Numerical aberrations				
+X	0	1	2	0
-X	1	2	1	1
+Y	0	0	0	1
-Y	3	3	4	0
Structural aberrations				
del(Xq)	0	0	0	1
Total no. patients[a]	4	5	6	2

[a] Some patients had more than one abnormality.



CASE 2. Papillary Carcinoma

Figure 1 Karyotype from case 2, papillary carcinoma: 49,X, -Y,der(2)t(1;2)(q25;q37), + del(6)(q13), +7, +7,der(15) t(3;15)(q11;p11), + 20.

DISCUSSION

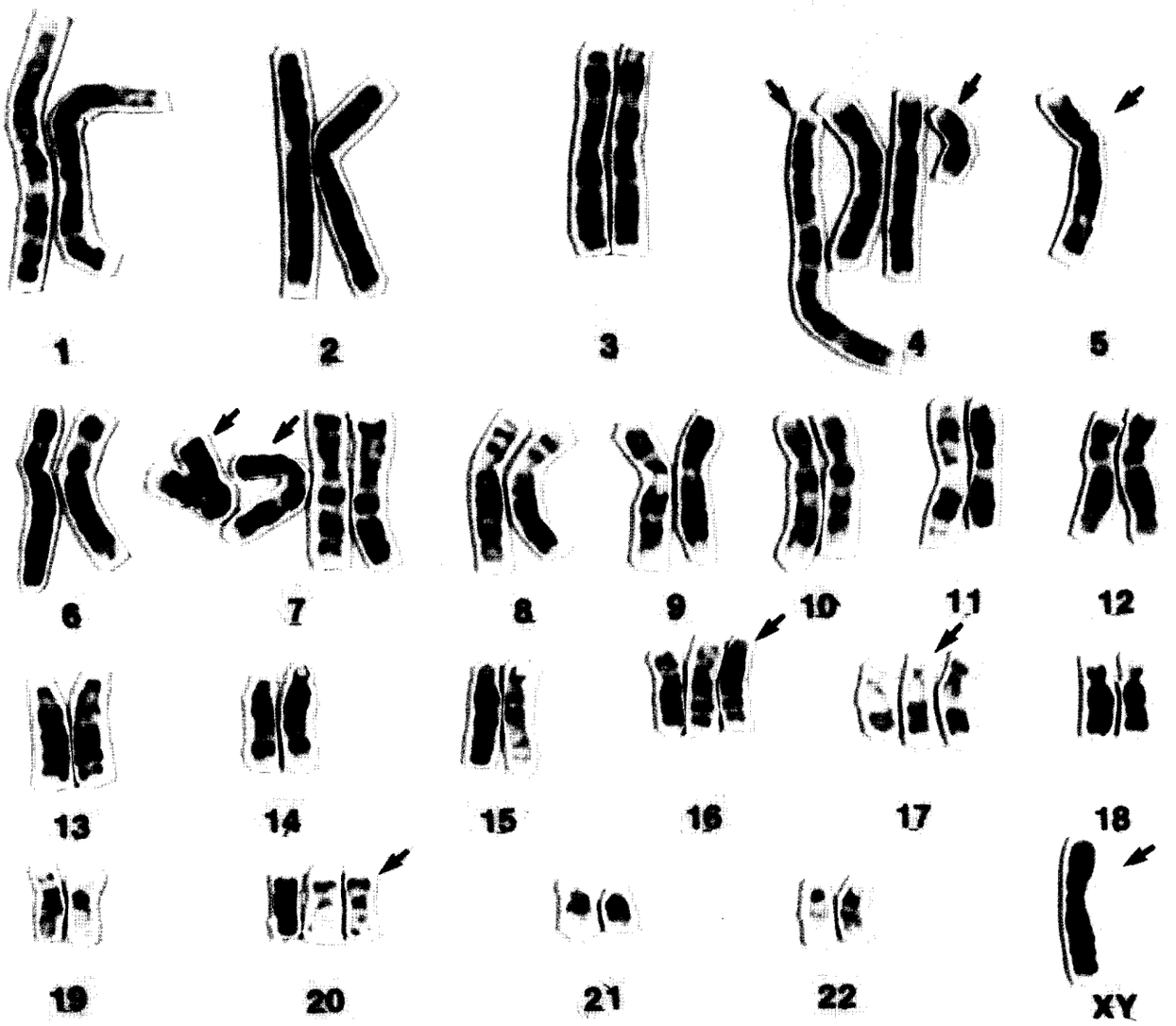
In the present study, utilizing both short- and long-term cell culture, we studied the cytogenetic characteristics of 25 patients with nonfamilial renal cell carcinoma. This study was undertaken to determine whether or not specific chromosomal abnormalities correlated with different histologic subtypes of renal tumors. Our results demonstrated that numerical and structural aberrations most frequently involved chromosomes 1, 3, 7, 9, and the Y.

Chromosome defects with del(3)(p14–p23) and LOH have been noted in a variety of cancers, including benign pleomorphic adenomas of the salivary gland in the form of t(3;8)(p21;q12) [17], and most notably in small cell and non-small cell lung cancer [18–20]. Chromosome 3p abnormalities resulting in loss of DNA sequences appear to also play a critical role in the development of renal cell carcinoma [5–10] and have been observed in up to 75% of cases [21]. As revealed by cytogenetic analysis, the location of the breakpoint has ranged from 3p11 to 3p23, clustering around 3p13–14, and RFLP analysis has shown loss of alleles in this segment in most RCCs. The cytogenetic and molecular evidence in RCC are consistent with the hypothesis that a "recessive cancer gene" or "cancer suppressor gene" resides on 3p and that loss of this gene facilitates the development of RCC,

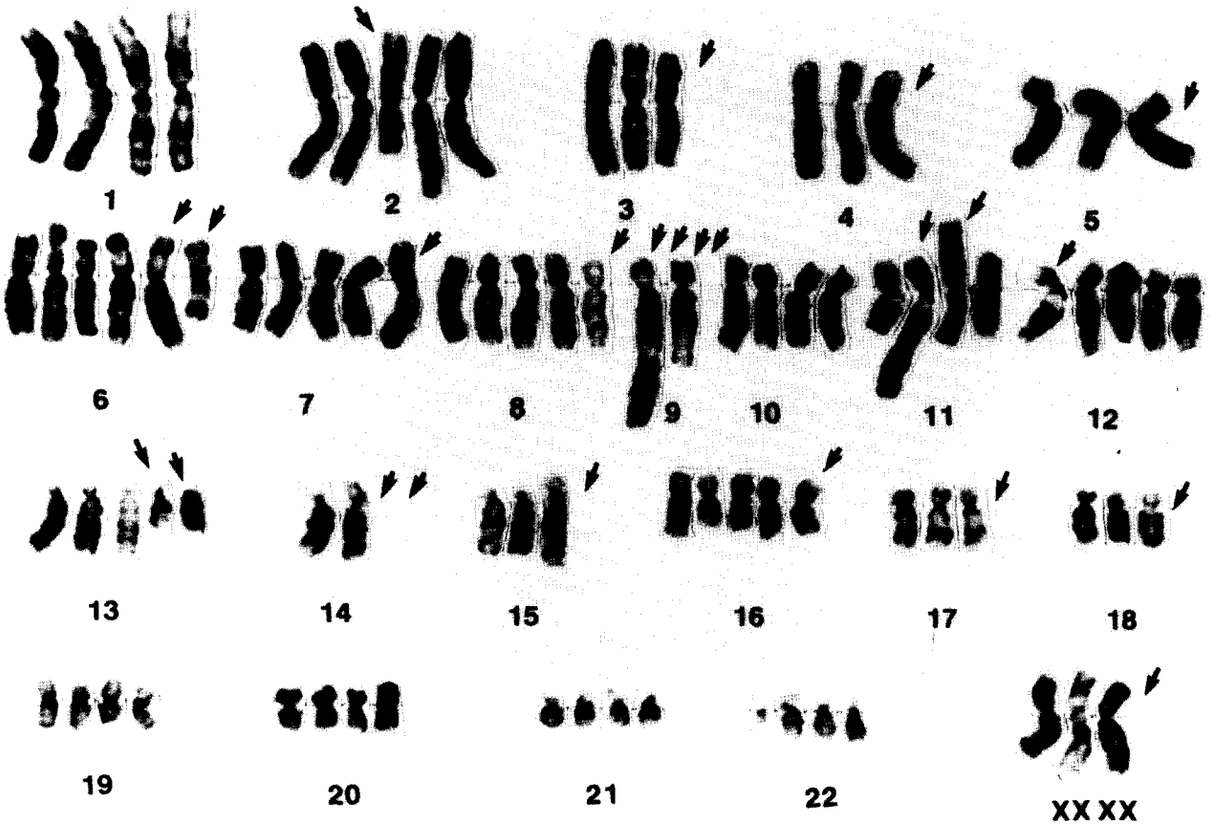
akin to the two-hit process described by Knudson for retinoblastoma [22]. In support of this the von Hippel-Lindau (VHL) tumor suppressor gene was recently identified [23] and shown to be reduced to homozygosity in 98% of sporadic, nonpapillary renal carcinomas and contain somatic mutations in nearly 60% of nonpapillary tumors [24]. Therefore, it is likely that coupled deletion and mutation of VHL is the major initiating event in nonpapillary renal tumorigenesis.

Although few studies have reported the histologic subtype of RCC, Kovacs et al. [12] determined by both cytogenetic analysis and DNA studies that 3p loss is restricted to the common nonpapillary RCCs and that it never occurs in papillary RCC. Similarly, in a report of nine renal tumors, Carroll et al. [10] reported clonal abnormalities affecting 3p12–21 in five of six clear cell tumors but no changes of 3p in two cases of papillary (one tubular-papillary and one acinarpapillary) carcinoma. Maloney et al. [25] found a somewhat lower incidence of 3p involvement, 52% (11/21 cases), in their cases of clear and granular/clear RCC. In their 40 cases of renal tumors, Meloni et al. [26] found monosomy 3 or 3p rearrangement in 40% of their 32 nonpapillary cases (including 15 cases of renal cell carcinoma, 14 clear cell RCC, one granu-

Figure 2 Karyotype from case 17, papillary carcinoma: 51,X, −Y, + der(4)t(4;7;11;5)(:4p12→4q13::7p11→7pter::11q13→11q25::5q13→5qter), + i(4p), − 5, + 7, + 7, + 16, + del(17)(p12), + 20.



Case 17. Papillary Carcinoma



Case 5. Clear Cell Carcinoma

Figure 3 Karyotype from case 5, clear cell carcinoma: 89,XXX, – X, + del(2)(q31), – 3, – 4, – 5, + 6, + i(6p), + 7, + 8, – 9, – 9, der(9)t(1;9)(p11;q11),der(9)t(2;9)(q11;q34),der(11)t(1;11)(q22;q25),der(11)t(8;11)(q11;p11), + der(12)(1;12)(q21;q13), + 13,del(13)(q21)x2, – 14, – 14 – 15, + 16, – 17, – 18.

Table 3 Chromosome 1 aberrations in RCC

	Papillary	Clear	Clear/granular	Clear/granular/ sarcomatoid
Total No. Pts./ Subtype	7	8	7	3
Numerical aberrations				
gain (+)	0	1	1	0
loss (–)	0	1	0	1
Structural aberrations				
Deletions				
p arm	1	0	0	1
q arm	1	2	2	1
p & q arms*	1	0	0	0
isochrom.	1	0	0	0
Translocations	3	1	0	1
Total no. pts.** with abnormality	6	5	3	3

* Not the same chromosome.

** Some patients had more than one abnormality.

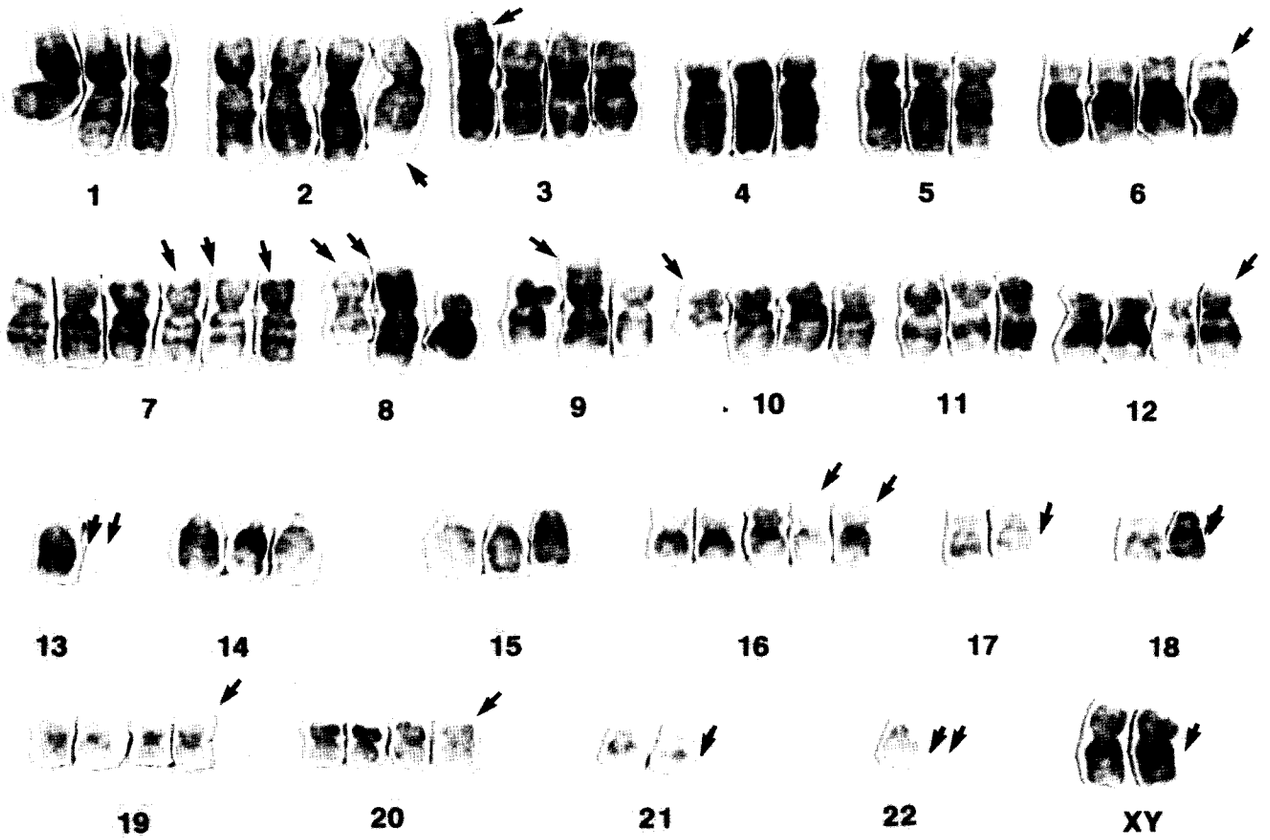


Figure 4 Karyotype from case 9, clear/granular cell carcinoma: 73,XX, −Y, +2, +der(3)t(3;7)(p21;q11), +6, +7, +7, +7,i(8p),i(8q),i(9q), +del(10)(q22), +12, −13, −13, +16, +16, −17, −18, +19, +20, −21, −22, −22.

lar RCC, one glomerular RCC, and one Wilm's tumor); one of their eight papillary RCCs had a 3p12 rearrangement. The incidence in the present study is similar to those reported in the latter two studies: in our patients, cytogenetic evidence of 3p structural involvement or numerical loss occurred in a total of 10 of 18 (55.6%) nonpapillary patients. Structural aberrations of 3p, either in the form of deletions or translocations, were seen in eight of the 15 patients (53.3%) with clear or clear/granular RCC and one of three patients with clear/granular/sarcomatoid RCC. Of the seven papillary pa-

Table 4 Chromosome 3 aberrations in RCC

	Papillary	Clear	Clear/granular	Clear/granular/sarcomatoid
Total no. pts./subtype	7	8	7	3
Numerical aberrations				
Gain (+)	1	2	0	1
Loss (−)	1	2	1	0
Structural aberrations				
Deletions				
p arm	1	2	1	1
q arm	0	1	0	0
p & q arm	0	0	0	1
inv q arm	0	1	0	0
Translocations				
Involving chromosome 7	0	1	2	0
Involving other chromosomes	1	1	2	1
Total no. patients[a]	4	6	5	2

[a] Some patients had more than one abnormality.

Case 14. Clear/Granular Cell Carcinoma

Figure 5 Karotype from case 14, clear/granular cell carcinoma: 50,X, -Y, +1, +1,der(3)t(3;8)(p14;q13), +5, +7, +9, -10, -10, +del(11)(q13), +12, +del(13)(q14)x2, -14, -17, +20, -21.

tients, one had a 3p deletion in a lymph node metastatsis which was not seen in the primary tumor. Loss of chromosome 3 was seen in three of the clear or clear/granular patients (two of whom also had 3p structural aberrations) and one of the seven papillary patients; in the latter patient, however, it was difficult to ascertain since the number of chromosome 3s varied considerably from cell to cell. We concluded, therefore, that our data, including the clustering of

Table 5 Chromosome 7 aberrations in RCC

	Papillary	Clear	Clear/granular	Clear/granular/sarcomatoid
Total no. pts./subtype	7	8	7	3
Numerical aberrations				
Gain (+)	5	4	4	2
Loss (-)	0	0	0	0
Structural aberrations				
Deletions				
p arm	0	0	0	0
q arm	2	1	0	1
iso (7q)	0	1	0	0
Translocations	3	2	2	2
Total no. patients[a]	6	6	5	3

[a] Some patients had more than one abnormality.

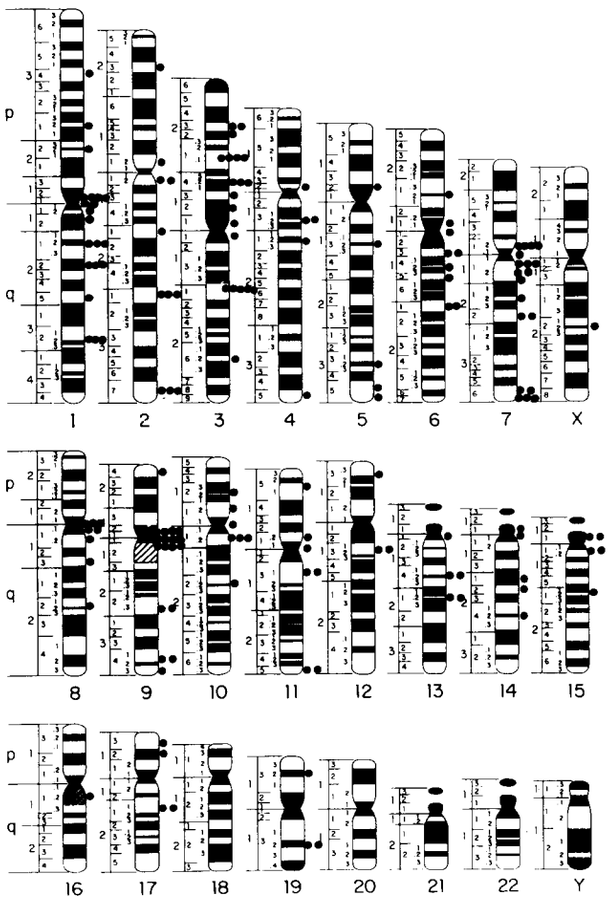


Figure 6 Chromosome idiogram showing breakpoints for each structural abnormality observed in 27 cases of renal cell carcinoma (RCC).

breakpoints at 3p13–p21, support the findings of previous studies.

Molecular studies of loss of heterozygosity (LOH) of 3p using RFLP analysis have been done in both sporadic and hereditary RCC. Cohen et al. [3] described a family with a constitutional t(3;8)(p21;q24) where all translocation carriers developed renal cancer; the breakpoint was subequently shown to be at 3p14.2 [27] and molecular studies [28] using three probes confirmed that two probes, pH3E4/D3S48 and pHF12–32/D3S2, were confined to 3p21, distal to the breakpoint in 3p and that a third probe, pMS1–37/D3S3, localized close to the breakpoint at 3p14. In two papers, Anglard et al. [15, 29] found LOH in 88% (51/58) of RCC patients at one or more of 10 loci independent of tumor stage, with the distal portion of 3p bounded by D3S2 and D3S22 (3p21–26) as the region of the disease gene. LOH was similarly seen in 89% (25/28) of nonpapillary RCC cell lines. That report found no correlation between LOH and clear or granular cell phenotypes. Zbar et al. [11] and Kovacs et al. [30] found that one allele of the pH3H2 (D1S1) DNA probe, which maps distal to the most common breakpoint, is deleted in most RCCs. And in a study of LOH in subtypes of RCC, Kovacs et al. [12] found LOH for the D3F15S2 locus and for one allele of the THRB and the RAF-1 genes in nonpapillary RCC, but retention of both alleles in papillary RCC.

A summary of our 3p cytogenetic and deletion analyses is shown in Table 7. With the exception of metastatic tumor in patient 16, structural chromosome 3p abnormalities were not seen in papillary tumors. However, evidence of 3p cytogenetic abnormalities and deletions were in agreement in the nonpapillary patients. There did not appear to be any association between cytologic subtype and 3p abnormalities. Therefore, it is possible that the tumor cytology is independent of the initial tumorigenic event, chromosome 3p deletion, and mutation of the VHL gene.

Chromosome 7 was most frequently involved in chromosomal aberrations in our RCC patients: 20 of 25 patients had chromosome gain (trisomy or tetrasomy 7 in 15 patients) and/or structural abnormalities (17 patients), with breakpoints clustering around the centromere and the terminal portion of 7q. Little difference was seen in the incidence of chromosome 7 involvement in the various disease subtypes, and in none of our patients was trisomy seen as the sole abnormality. A gain of chromosome 7 has frequently been reported in RCC, either as the sole cytogenetic change or in combina-

Table 6 Isochromosome aberrations in RCC

	Papillary	Clear	Clear/granular	Clear/granular/sarcomatoid
Total no. pts./subgroup	7	8	7	3
i(1q)	1	0	0	0
i(4p)	1	0	0	0
i(6p)	0	1	0	0
i(7q)	0	1	0	0
i(8q)	1	0	0	0
i(8p), + i(8q), + i(9q)	0	0	1	0
i(8q), + i(9q)	0	0	1	0
i(8q), + i(9p), + i(11q)	0	1	0	0
i(9p), + i(9q)	0	0	0	1
i(10q)	0	0	1	0
i(14q)	1	0	0	0
Total no. patients	4	4	2	1

Table 7 Comparison of chromosome 3p loss of heterozygosity and chromosome 3 karyotypic abnormalities[a]

Tumor no.	Name	Histology[b]	3p14.2–p21 D3S2	3p21 D3S32	3p21 D3F15S2	3p24 THRB	3p24–25 D3S588	3p25 RAF1	3p25.5 VHL	3p26 D3S18	3p Abnormality
8	UOK115	Cl			●	●	●	–	●	–	del(3)(p11p22)
10	UOK101	Cl						●	●	–	+3
11	UOK116	Cl			●	–	●	●		●	t(3;7)(p23;q36), +del(3)(p23)
15	UOK107	Cl	●	●	–	–		–	○		inv(3)(q21q26)
9	UOK122	Cl/gr			–	–	●	–	●	–	+t(3;7)(p21;q11)
12	UOK113	Cl/gr		●	–	–		●		–	+t(3;7)(p21;q26)
22	UOK117	Cl/gr			●	●	●	●	●	–	del(3)(p14p23)
23	UOK121	Cl/gr/sarc			–	–	●	–	–		None
24	UOK130	Cl/gr/sarc			●	●	–	–	●		del(3)(p14q21)t(3;4), (p13;q35)del(3)(p13)
1	UOK112	Papillary	○		–	–	○	○	–	–	None
3	UOK124	Papillary	–		–	–		○	–	–	None
16	UOK109	Papillary	–		○	–		–	○	○	None
16 met	UOK109LN	Papillary	–		○	–		–	○	○	+del(3)(p12)
21	UOK120	Papillary			○	–		–	○	○	+3

Abbreviations: ●, LOH; ○, retention of heterozygosity; –, tested but not informative. Blanks indicate untested loci.

[a] Chromosome 3p LOH at the indicated locus was previously demonstrated ([11] and Gnarra et al., in press).

[b] Histologic type was according to the Robson classification ().

tion with other abnormalities [31]. However, trisomy 7 has also been described in many other solid tumors, including those of lung, brain, bladder, ureter, and large bowel [32]. Weaver et al. [33], who found polysomy 7 to be the most frequent abnormality in their 26 RCC patients, suggested that abnormality of chromosome 7 represents a primary change, and that when it is associated with chromosome 3 abnormalities, it may be indicative of a more aggressive course and a higher stage of disease at diagnosis. The epidermal and platelet growth factor receptors are both located on chromosome 7 and several studies have indicated that there may be an association between chromosome 7 and cell proliferation, cell transformation, and acquisition of invasiveness [13, 14]. In a study of 30 RCCs, Limon et al. [34] found trisomy 7, either alone or in combination with 3p aberrations, in 17 tumors, but concluded that trisomy 7 does not represent a primary change nor should it be viewed as a tumor-specific abnormality.

In a study of 18 papillary renal cell tumors and a review of 20 previously published cases, Kovacs et al. [35] found that chromosomes 7, 16, 17, and the Y were specifically involved in this type of RCC; these tumors had neither 3p nor 5q rearrangements. They also studied 10 benign papillary renal cell adenomas and concluded that a combination of tri- and tetrasomy 7 and trisomy 17 (the only autosomal changes) were markers for this type of adenoma; loss of the Y was seen in seven of the nine male patients. Our papillary RCC cases showed somewhat different results: five of our seven patients had +7, four had –Y, three had +16, and only one of the seven cases had +17. Two other reports in the literature also support the conclusion that +7, +17, and –Y are not specific to papillary RCC. Dal Cin et al. [36] examined three cortical tumors (adenomas) of the kidney and found +7, +7, +17, and –Y was present in all cases; no 3p or 5q aberrations were observed. None of the three cases had features of papillary RCC and all had large primary renal tumors without metastases. In their 40 renal cases with clonal abnormalities, Meloni et al. [26] observed +7, +17, and –Y in almost all RCC subtypes studied, including RCC, clear cell, papillary, granular, and Wilm tumor. They also concluded that chromosome 7 may play a role in cell proliferation.

Abnormalities of chromosome 1 are the most common rearrangements seen in neoplasia; however, no specific breakpoint has been associated with any particular disease. Few chromosome 1 abnormalities have been reported in RCC. A total of 17 of our patients had numerical or structural chromosome involvement (Fig. 6), but no specific abnormality or breakpoint was noted.

Sex chromosome loss is a prominent feature of RCC, loss of the Y being more common than loss of the X. Kovacs and Frisch [37] reported –Y in 85% of papillary and 30% of nonpapillary RCCs. Loss of the Y has been observed in other neoplasms and is commonly seen in the bone marrow of normal aging males [38]. Limon et al. [34] noted sex chromosome loss, primarily –Y, in 14 of their 30 RCC tumors, and as the sole change in five tumors. Kovacs and Frisch concluded that their results suggested a nonrandom involvement of the Y chromosome in papillary RCC, with perhaps involvement of the homologous regions of X and Y, the site of a possible suppressor gene. Our results demonstrated that 52% of our patients showed sex chromosome loss, with 10/22 males showing –Y (four of our seven papillary cases had –Y). Limon et al. concluded that, like trisomy 7 (discussed above), sex chromosome loss should not be regarded as a primary change or a tumor-specific abnormality in RCC. Meloni et al. [39] reported a unique translocation, t(X;1)(p11.2;q21), in four male patients with papillary renal tumors. They concluded that these cases represent a separate subtype of renal tumors, and suggested that since all cases were males that the X chromosome contains one or more tumor suppressor genes. This translocation was not observed in any of our cases.

Two of our cases (one clear cell and one clear/granular) had t(3;5)(p21;q33 or q35). Rearrangement of chromosome 5 was observed in 13 of 25 RCC tumors by Kovacs et al. [9], with all but one translocation involving 5q to 3p13; the breakpoint on 5q was variable, with the shortest region of overlap being 5q22–qter in 12 of 25 cases. The result of the translocation was a polysomic state for 5q22–qter. Although the breakpoint in Kovacs et al.'s cases was proximal to the proto-oncogene *FMS*, located at 5q34, the breakpoint in our two cases was at this band. The significance of 5q involvement, however, remains to be elucidated.

Eleven of our cases had isochromosome formation, the most frequent being i(8q), which was seen in four patients. According to a recent review of isochromosomes in neoplasia by Mertens et al. [40], 7.8% of 410 reported cases of cytogenetically abnormal kidney tumors demonstrated isochromosome formation, the most frequent being i(1q) (11 cases) and i(8q) (seven cases). Isochromosome 8q was the second most common isochromosome in the almost 20,000 neoplasms gathered from the literature; it is a recurrent abnormality in almost all tumor types, most notably in lymphocytic leukemias, lymphomas, and adenomas of the lung, colon, and stomach. It is considered to be a secondary aberration and it is thought that it is the gain of 8q rather than the loss of 8p which confers proliferative advantages to the cell. The highest frequency (17%) of i(8q) was found in malignant melanoma, especially the uveal type, where it is associated with monosomy 3 (18/21 cases with i[8q] had −3; none of our four patients with i[8q] had −3).

In summarizing our results in 25 patients with RCC, we observed that chromosomes 7, 3, 1, 9, and the Y (in descending order of frequency) were most involved in numerical and structural abnormalities. Polysomy of chromosome 7 was the most frequent numerical abnormality, followed by loss of the Y. Chromosome 3 was most involved in structural abnormalities, while showing numerical loss as well, and two of our clear cell patients had t(3;5) (p21;q34). Our findings support those of previous studies; the incidence of 3p involvement in nonpapillary RCC, whether numerical or structural, was not as high as reported in several studies by Kovacs and colleagues, although in accord with the reports of Moloney et al. and Meloni et al. The significance of 3p abnormalities in RCC appears to warrant further study, as does the true significance of polysomy 7 and loss of the sex chromosomes, which may not be specific to RCC. Other than 3p changes in nonpapillary RCC, we found no specific abnormalities associated with any particular subtypes of RCC.

REFERENCES

1. Frank IN, Graham SD Jr, Nabos WL (1991): Urologic and male genital cancer. In: American Cancer Society Textbook of Clinical Oncology, 1st Ed., AI Holleb, DJ Fink, GP Murphy, eds. American Cancer Society, Atlanta, pp. 271–276.
2. Dekernion JB, Lowitz BB, Casciato DA (1991): Urinary tract cancers. In: Manual of Clinical Oncology, 2nd Ed., DA Casciato, BB Lowitz, eds. Little, Brown and Company, Boston, p. 198.
3. Cohen AJ, Li FP, Berg S, Marchetto DJ, Tsai S, Jacobs SC, Brown RS (1979): Hereditary renal-cell carcinoma associated with a chromosomal translocation. N Engl J Med 310:592–595.
4. Pathak S, Strong LC, Ferrell RE, Trindade A (1982): Familial renal cell carcinoma with a 3;11 chromosome translocation limited to tumor cells. Science 217:939–941.
5. Jordan DK, Divelbiss JE, Waziri MH, Burns TL, Patil SR (1989): Fragile site expression in families with von Hippel-Lindau disease. Cancer Genet Cytogenet 39:157–166.
6. Berger S, Sandberg AA, Todd IAD, Pennington RD, Haddad FS, Hecht BK, Hecht F (1986): Chromosomes in kidney, ureter, and bladder cancer. Cancer Genet Cytogenet 23:1–14.
7. Yoshida MA, Ohyashiki K, Ochi H, Gibas Z, Pontes JE, Prout GR, Sandberg AA (1986): Cytogenetic studies of tumor tissue from patients with nonfamilial renal cell carcinoma. Cancer Res 46:2139–2147.
8. Teyssier JR, Henry I, Dozier C, Ferre D, Adnet JJ, Pluot M (1986): Recurrent deletion of the short arm of chromosome 3 in human renal cell carcinoma: Shift of the c-raf-1 locus. J Natl Cancer Inst 77:1187–1195.
9. Kovacs G, Szucs S, Riese WD, Baumgartel H (1987): Specific chromosome aberration in human renal cell carcinoma. Int J Cancer 45:197–205.
10. Carrol PR, Murty VVS, Reuter V, Jhanwar S, Fair WR, Whitmore WF, Chaganti RSK (1987): Abnormalities at chromosome region 3p12–14 characterize clear cell renal carcinoma. Cancer Genet Cytogenet 26:253–259.
11. Zbar B, Brauch H, Talmadge C, Linehan M (1987): Loss of alleles of loci on the short arm of chromosome 3 in renal cell carcinoma. Nature (London) 327:721–724.
12. Kovacs G, Wilkens L, Papp T, DeRiese W (1989): Differentiation between papillary and nonpapillary renal cell carcinomas by DNA analysis. J Natl Cancer Inst 81:527–530.
13. Merlino GT, Oshii S, Whang-Peng J, Knutsen T, Xu Y-H, Clark AJL, Stratton RH, Wilson RK, Ma DP, Roe BA, Hunts JH, Shimizu H, Pastan I (1985): Structure and localization of the genes encoding the aberrant and normal epidermal growth factor receptor RNAs from A431 human carcinoma cells. Mol Cell Biol 5:1722–1734.
14. Walter TA, Berger CS, Sandberg AA (1989): The cytogenetics of renal tumors. Where do we stand, where do we go? Cancer Genet Cytogenet 43:15–34.
15. Anglard P, Trahan E, Liu S, Latif F, Merino MJ, Lerman MI, Zbar B, Linehan WM (1992): Molecular and cellular characterization of human renal cell carcinoma cell lines. Cancer Research 52:348–356.
16. ISCN (1991): Guidelines for Cancer Cytogenetics, Supplement to an International System for Human Cytogenetic Nomenclature. Mitelman F (ed), Karger, Basel.
17. Mark J, Dahlenfors R (1986): Cytogenetical observations in 100 human benign pleomorphic adenomas: Specificity of the chromosomal aberrations and their relationship to sites of localized oncogenes. Anticancer Res 6:299–308.
18. Whang-Peng J, Kao-Shan CS, Lee EC, Bunn PA, Carney DN, Gazdar AF, Minna JD (1982): A specific chromosome defect associated with human small cell lung cancer: deletion 3p(14–23). Science 215:181–182.
19. Whang-Peng J, Knutsen T, Gazdar A, Steinberg SM, Oie H, Linnoila I, Mulshine J, Nau M, Minna JD (1991): Non-random structural and numerical changes in non-small cell lung cancer. Gene Chrom Cancer 3:168–188.
20. Brauch H, Johnson B, Hovis J, Yano T, Gazdar A, Pettengill OS, Graziano S, Sorenson GD, Poiesz BJ, Minna J, Linehan M, Zbar B (1987): Molecular analysis of the short arm of chromosome 3 in small-cell and non-small-cell carcinoma of the lung. NEJM 317:1109–1113.
21. Teyssier JR, Ferre D (1990): Chromosomal changes in renal cell carcinoma cell. No evidence for correlation with clinical stage. Cancer Genet Cytogenet 45:197–205.
22. Knudson AG (1987): A two mutation model for human cancer. Adv Viral Oncol 7:1–17.

23. Latif F, Tory K, Gnarra J, Yao M, Duh F-M, Orcutt ML, Stackhouse T, Kuzmin I, Modi W, Geil L, Schmidt L, Zhou F, Li H, Wei MH, Chen F, Glenn G, Choyke P, Walther MM, Weng Y, Duan D-SR, Dean M, Glavac D, Richards FM, Crossey PA, Ferguson-Smith MA, Le Paslier D, Chumakov I, Cohen D, Chinault AC, Maher ER, Linehan WM, Zbar B, Lerman MI (1993): Identification of the von Hippel-Lindau disease tumor suppressor gene. Science 260:1317–1320.
24. Gnarra JR, Tory K, Weng Y, Schmidt L, Wei MH, Li H, Latif F, Liu S, Chen F, Duh F-M, Lubensky I, Duan DR, Florence C, Pozzatti R, Walther MM, Bander NH, Grossman HB, Brauch H, Pomer S, Brooks JD, Isaacs WB, Lerman MI, Zhar B, Linehan WM (1994): Mutations of the VHL tumor suppressor gene in renal carcinoma. Nature Genet 7:85–90.
25. Maloney KE, Norman RW, Lee CLY, Millard OH, Welch JP (1991): Cytogenetic abnormalities associated with renal cell carcinoma. J Urology 146:692–696.
26. Meloni AM, Bridge J, Sandberg AA (1992): Reviews on chromosome studies in urological tumors. I. Renal tumors. J Urol 148:253–265.
27. Wang N, Perkins KL (1984): Involvement of band 3p14 in t(3:8) hereditary renal cell carcinoma. Cancer Genet Cytogenet 11:479–481.
28. van der Hout AH, Brown RS, Li FP, Buys CHCM (1991): Localization by in situ hybridization of three 3p probes with respect to the breakpoint in a t(3;8) in hereditary renal cell carcinoma. Cancer Genet Cytogenet 51:121–124.
29. Anglard P, Trahan E, Liu S, Latif F, Merino MJ, Lerman MI, Zbar B, Linehan WM (1991): Molecular analysis of genetic changes in the origin and development of renal cell carcinoma. Cancer Res 51:1071–1077.
30. Kovacs G, Erlandson R, Boldog F, Ingvarsson S, Muller-Brechlin R, Klein G, Sumegi J (1988): Consistent chromosome 3p deletion and loss of heterozygosity in renal cell carcinoma. Proc Natl Acad Sci USA 85:1571–1575.
31. Wolman SR, Camuto PM, Golimbu M, Schinella R (1988): Cytogenetic, flow cytometric, and ultrastructural studies of twenty-nine nonfamilial human renal carcinomas. Cancer Res 48:2890–2897.
32. Mitelman F (1991): Catalog of Chromosome Aberrations in Cancer, Fourth Ed. Wiley-Liss, New York, pp. 575–681.
33. Weaver DJ, Michalski K, Miles J (1988): Cytogenetic analysis in renal cell carcinoma: Correlation with tumor aggressiveness. Cancer Res 48:2887–2889.
34. Limon J, Mrozek K, Heim S, Elfving P, Nedoszytko B, Babinska M, Mandahl N, Lundgren R, Mitelman F (1990): On the significance of trisomy 7 and sex chromosome loss in renal cell carcinoma. Cancer Genet Cytogenet 49:259–263.
35. Kovacs G, Fuzesi L, Emanuel A, Kung H (1991): Cytogenetics of papillary renal cell tumors. Genes Chrom Cancer 3:249–255.
36. Dal Cin P, Gaeta J, Huben R, Li FP, Prout GR, Sandberg AA (1989): Renal cortical tumors. Cytogenetic characterization. J Clin Pathol 92:408–414.
37. Kovacs G, Frisch S (1989): Clonal chromosome abnormalities in tumor cells from patients with sporadic renal cell carcinomas. Cancer Res 49:651–659.
38. Sandberg AA (1985): The Y chromosome in human neoplasia. In: The Y Chromosome, Part B: Clinical Aspects of Y Chromosome Abnormalities, AA Sandberg, ed. Alan R. Liss Inc., New York, pp. 377–393.
39. Meloni AM, Dobbs RM, Pontes JE, Sandberg AA (1993): Translocation (X;1) in papillary renal cell carcinoma. A new cytogenetic subtype. Cancer Genet Cytogenet 65:1–6.
40. Mertens F, Johansson B, Mitelman F (1994): Isochromosomes in neoplasia. Genes Chrom Cancer 10:221–230.